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The Influence of Bearing Cycles on Olive Oil Quality Response to Irrigation

Alon Ben-Gal,*[†] Arnon Dag,[‡] Loai Basheer,[§] Uri Yermiyahu,[†] Isaac Zipori,[‡] and Zohar Kerem[§]

[†]Institute of Soil, Water and Environmental Sciences and [‡]Institute of Plant Sciences, Gilat Research Center, Agricultural Research Organization, Mobile Post Negev 2, 85280, Israel

[§]Institute of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot, 76100, Israel

Ⓢ *Supporting Information*

ABSTRACT: Five rates of water application were applied in a 4 year study on olive (*Olea europaea*) varieties 'Barnea' and 'Souri'. Increased irrigation lead to increased tree-scale oil yields, lower polyphenol content, and, frequently, higher oil acidity. These effects were predominant in "off" years. The fatty acid profile was influenced primarily by bearing level and variety and secondarily by irrigation rate. The saturated to unsaturated fatty acid ratio was higher in "off" than in "on" years, and the monounsaturated fatty acid to polyunsaturated fatty acid ratio was higher in "on" years as a result of the fact that oleic and stearic acids were higher in "on" years, while palmitic, palmitoleic, and linoleic acids were greater in "off" years. Squalene was higher in 'Souri' than in 'Barnea' oils, was not affected by bearing cycle, and was consistently lower in oil from trees receiving the lowest irrigation level.

KEYWORDS: *Olea europaea*, water application, fruit load, free fatty acids, polyphenols, fatty acid profile, squalene

INTRODUCTION

Olive production has historical importance throughout the Mediterranean. Traditionally, olives are not irrigated, but recently, water application has been recognized as constructive in order to (a) increase yields and the quality of olives in regions with traditional rain-fed olive production, (b) allow high-density olive orchards, and (c) expand olive production into regions where there is not enough rainfall to otherwise support the crop.[1] The literature shows that under typical Mediterranean climatic conditions (hot and dry summers), irrigation can promote increased olive fruit and oil yields by as much as 4-fold.[2−6] This impressive yield response to water application can be due to a number of factors. First is that irrigation allows avoidance of conditions of stress that would otherwise cause reduced olive oil accumulation in fruits.[7,8] A second, probably more important, reason that irrigation increases yields is an augmented capacity for quantity of fruit per tree.[9] However, increased yields may come at a price in terms of oil quality. Irrigation has been found to decrease polyphenol content[10−18] and, consequently, the bitter index and oxidative stability of the oil.[16,13,10,11,18] The few cases where the opposite trend, increased polyphenol content with increased irrigation, has been reported[19,20] are likely explained due to the fact that irrigated trees were harvested at lower ripeness levels as compared to the nonirrigated ones. Most irrigation studies in olives show no effect on the major quality criteria: free fatty acids (FFAs) content and peroxide value.[11,21,18,22] However, there have been a number of reports where irrigation negatively affected FFA content.[23,15,24]

Gomez-Rico et al.[13] found a significantly higher oleic acid content and monounsaturated fatty acid (MUFA)/polyunsaturated fatty acid (PUFA) ratio in oils obtained from rain-fed olives as compared to oils from irrigated olives. In spite of this, the bulk of the literature suggests that irrigation has little to no effect on olive oil fatty acid composition.[25,16,11,21]

Olive has a strong tendency for alternate bearing. Being an industry-dependent commodity, the economic problems arising from this behavior are of particular concern.[26] While the phenomenon has been significantly reduced in moderate-yielding orchards, high-yielding oil olive cultivars such as 'Barnea' are particularly prone to alternate bearing. In extreme cases, an intensively cultivated 'Barnea' grove fluctuates between 90 and 10 kg of fruit per tree or between 18 and 2 kg of oil per tree in successive "on" and "off" years, respectively.[27] Physiologically, biennial bearing in olive is thought to be induced by production of secondary metabolites in developing seeds and their excretion and movement to stems and leaves where they inhibit floral bud induction and differentiation.[26] Lately, it has been established that one of the main causes for biennial bearing is the limitation of flowering sites following an "on" year.[28] In addition, resources consumed during the metabolic effort required by heavily yielding trees during oil production late in the season might come at the expense of subsequent reproductive processes.[29]

In spite of the many studies that have explored water stress and olive oil characteristics, there is little known regarding the effect of fruit load on oil quality and even less regarding combined effects of water stress and fruit load. The objective of this study was therefore to determine the combined effects of irrigation level and fruit load on oil quality.

MATERIALS AND METHODS

Research was conducted in a 2 hectare section of a 4 year old commercial olive orchard located (31°75′N, 34°85′E) adjacent to Kfar

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Menachem and Revadim in the foothills of the Judean Hills in Israel. The orchard section included both 'Souri' and 'Barnea' varieties where between every four rows of 'Barnea', there were two rows of 'Souri'. Orchard design and spacing was to accommodate mechanized harvesting by trunk shakers. Trees were planted every 3.75 m ('Souri') and 4.25 m ('Barnea') in rows spaced 7 m apart. Irrigation application for each treatment was determined as a fraction of the daylight-hour reference evapotranspiration (ET_0) calculated using the Penman−Monteith equation.[30] Meteorological data were collected from a station located adjacent to the orchard. Five irrigation levels (30, 50, 75, 100, and 125%) were given. Actual daily irrigation (I) was computed by:

$$I = \mathrm{ETp} \times \frac{\mathrm{IrrLev}}{100}\ ; \mathrm{ETp} = \mathrm{ET}_0 \times f_c \qquad (1)$$

where ETp is potential evapotranspiration, IrrLev is the level of irrigation for the different treatments (%), and f_c is the cover factor estimated by midday shaded area. The IrrLev fraction is equivalent to a crop factor (Kc) as found in FAO crop water consumption methods.[31] The cover factor, f_c, was estimated as 40% in 2006 and 50% from 2007 to 2009. Statistical design was randomized complete-block with five replicates per treatment. Each of the 25 experimental units consisted of six adjacent tree rows (four 'Barnea' and two 'Souri') with at least four olive trees per row. Two central trees of each variety were monitored while those surrounding served as border-guard trees. Detailed site and experimental descriptions are found in ref 9.

Yield was determined for each monitored tree, harvested at the approximated appropriate ripeness level (50% black fruit, maturity index = 3.5). Details of tree growth, water stress levels, and fruit yield were previously published.[9] The oil percentage was measured by chemical extraction with Soxhlet using *n*-hexane. Cold-pressed virgin oil was obtained with an Abencor system (MC2 Ingenieria y Sistemas, Spain) as described by Ben-David et al.[32] Chemical quality parameters tested on the oil included the following: FFA, peroxide value, and total polyphenols. Determination of FFA and peroxide value was carried out following the analytical methods described in ISO (International Organization for Standardization) 660 and 3960, respectively. FFA (ISO 660), given as a percentage of oleic acid, was determined by titration of a solution of oil in ethanol/ether (1:1) with ethanolic potassium hydroxide. Peroxide value (ISO 3960), expressed in milliequivalents of active oxygen per kilogram of oil ($\mathrm{mequiv\ kg^{-1}}$), was determined as follows: A mixture of oil and isooctane:acetic acid 3:2 was left to react in darkness with a potassium iodide solution, and the free iodine was then titrated with a sodium thiosulfate solution. Phenolic compounds were isolated from a solution of oil in hexane by triple extraction with methanol−water (60:40, V:V). Total phenols, expressed as tyrosol equivalents (ppm), were determined with a UV visible spectrophotometer (Beckman) at 735 nm using the Folin−Ciocalteu reagent.[33] Organoleptic analysis, providing quantitative description of the main positive as well as negative attributes of an oil, was conducted by a trained and authorized tasting panel according to the European Commission for Classifying Oils (Appendix II of EU regulation no. 2568/91).

The fatty acid profile of the oil was determined by gas chromatography (GC) after methyl esterification of the acids. The samples were methylated after alkaline hydrolysis using the $\mathrm{BF_3/MeOH}$ method according to AOCS protocol no. Ce 2-66.31. Chromatographic analysis was performed in an Agilent Technologies GC (model 6890N) equipped with a flame ionization detector and a Quadrex column (60 m long, 0.25 mm in diameter, 0.25 μm film thickness) (Quadrex Corp., Woodbridge, CT). Helium was employed as the carrier gas at a flow rate of 1 mL/min. The temperatures of both the injector and the detector were set at 250 °C, and the oven temperature was set at 210 °C. The injection volume was 1 μL (regulation EEC 2568/91, corresponding to AOCS method Ch 2-91; EEC, 1991). Fatty acids were identified by comparing retention times with those of standard compounds. The relative composition of the fatty acids in the oils was determined as percentage of total fatty acids. To simplify the discussion of the results, only the main fatty acids, palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3), are presented. A novel method was used to determine squalene level in oils. Peaks caused by known squalene concentrations were identified in fatty acid methyl ester (FAME) chromatograms and used to build calibration curves. Squalene was subsequently determined in experimental samples along with fatty acids.

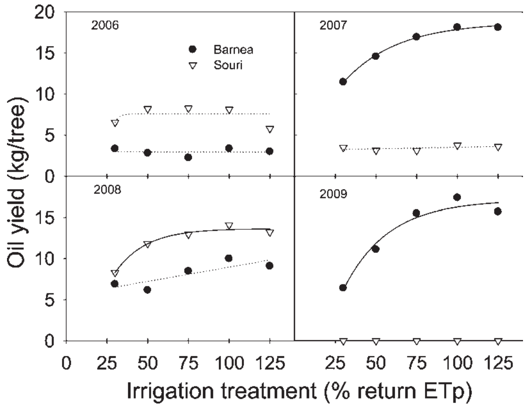

Figure 1. Olive oil yield as a function of irrigation water application rate. Symbols are average measured values ($n = 10$), and lines are best-fit three-parameter exponential rise to maximum or linear regression curves. Dotted lines are not significant. Regression equations and statistics are found in the Supporting Information, Tables A, D, and E. Reprinted with permission from ref 9. Copyright 2011 Springer.

FFA, peroxide number, total polyphenol content, and fatty acid composition were analyzed by two-way analysis of variance with JMP 5.0 software (SAS Institute Inc., United States). Relationships between the irrigation levels and the measured parameters were determined using regression analysis with SigmaPlot 11.0 (Systat Software, San Jose, CA). Default significance levels were set at $\alpha = 0.05$.

■ RESULTS

Yields. The orchard's two cultivars were in opposite biennial bearing cycles throughout the reported experimental years. In 2006 and 2008, 'Souri' was "on", with a high fruit load, and 'Barnea' was "off", while in 2007 and 2009, 'Barnea' was "on", and 'Souri' was "off". In the 2009 season, the 'Souri' trees were in a severe "off" year, and no fruit was harvested.

Oil yield was calculated as fruit yield multiplied by oil percentage (obtained by chemical extraction) and is shown in Figure 1. The oil percentage was higher in "on" than in "off" years and tended to decrease as a function of increased irrigation.[9] In the first year of the experiment, 2006, no effect of irrigation was found on oil yield. In the second year, 2007, significant increases of oil yield as a function of irrigation level were found for 'Barnea', which was in an "on" year, while no effect was seen for 'Souri' in an "off" year. In the third year of the experiment, 2008, significant increases of oil yield from 30 to 75% return rates were found for 'Souri' in an "on" year, and a nonsignificant trend of increased oil with increased water application was indicated for 'Barnea' in an "off" year. In the fourth year, 2009, the 'Souri' trees produced no fruit and therefore no oil, and 'Barnea' showed strong increases of oil as a function of increased irrigation until the 100% return treatment level. The oil yield was a function mostly of the

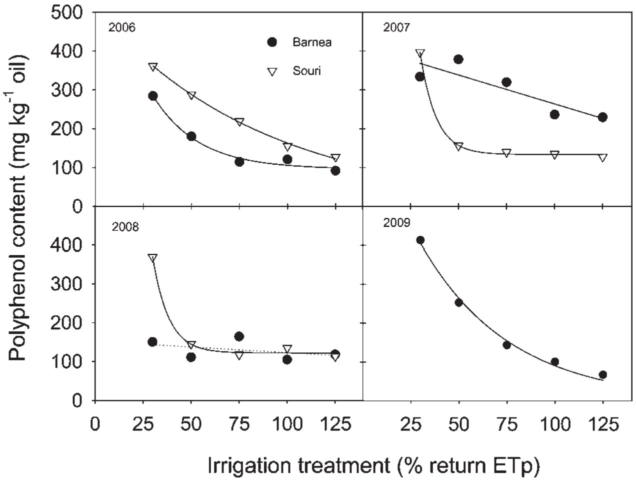

Figure 2. Polyphenol content of olive oil as a function of irrigation water application rate. Heavy fruit loads ("on" years) were experienced in 2006 and 2008 in 'Souri' trees and in 2007 and 2009 in 'Barnea'. Symbols are average measured values (n = 10), and lines are best-fit linear and one- or two-parameter exponential decay regression curves. Dotted lines are not significant. Regression equations and statistics are found in the Supporting Information, Tables A, D, and E.

number of fruits per tree although, for 'Barnea' in "on" years, oil per fruit was increased by irrigation.[9] Further results and discussion regarding tree and yield components and their response to the irrigation levels were published in a preliminary paper.[9]

Oil Quality. *Polyphenols.* The polyphenol content of oil was reduced as irrigation level increased (Figure 2). A very strong effect of irrigation on polyphenols was found for both varieties in 2006 and for 'Barnea' in 2007 and 2009. The lowest irrigation treatments produced oil with as high as 400 mg/kg total polyphenols, while oil from the highest irrigation treatments had polyphenols as low as 70 mg kg^{-1}. Oil from 'Souri' in both 2007 and 2008 showed very large decreases (from around 400 to around 150 mg kg^{-1} oil) as irrigation increased from 30 to 50% with only slight continued decreases with further irrigation. Oil from 'Barnea' in 2008 ("off" year) had low total polyphenols (between 100 and 150 mg kg^{-1}) with no significant effect of irrigation. Comparison of 2006 and 2007 data indicates a higher polyphenol content in oil from "on" as compared to "off" trees (Table 1).

FFAs. FFA levels were generally higher in "off" than in "on" years (Table 1). FFA levels in the oil increased as a function of increasing irrigation level for both cultivars in three of the four experimental seasons (Figure 3). The strongest effect was found in the first year of the experiment when high levels were found in oil from 'Barnea' in all but the lowest irrigation treatment, and exceptionally high (>2%) FFA levels were reached in oil produced from fruits receiving the highest irrigation treatment. FFA in oil from 'Souri' in 2006, 'Barnea' in 2009, and both varieties in 2008 similarly increased linearly with irrigation from around 0.3—0.4% at 30% ETp to 0.65—0.72% at 125% ETp. Results from 2007 were exceptional with no correlation between irrigation level and FFA values.

Peroxide. The peroxide level did not consistently respond to the irrigation level (Figure 4). The levels were generally low, between 4 and 8 mequiv O_2/ kg oil. Oil from 'Souri' trees in 2006 and 'Barnea' in 2009 had exponentially increasing peroxide from lowest irrigation plateauing at 75—100% treatment levels. The peroxide in oil from 'Souri' in 2007 decreased linearly as a function of increasing irrigation. None of the other season—cultivar combinations had any significant treatment related differences in peroxide level. The peroxide level did not appear to be consistently related to cultivar or fruit load (Table 1).

Fatty Acid Composition. The fatty acid composition was influenced strongly by variety and bearing level and less so by the irrigation treatments during the experiment period 2007—2009 (Figure 5). The average oleic acid ranged from 59 to 68% of total fatty acids, was consistently higher in 'Barnea' as compared to 'Souri' oils, and was greater in "on" as compared to "off" years (Table 1 and Figure 5a). The intermediate irrigation level treatment (75%) obtained the highest content of oleic acid in the years 2007 and 2009 in 'Barnea' oils and in the year 2008 in 'Souri' oils ("on" years). The percentage of linoleic acid was higher in "off" (17—18%) as compared to "on" (13—16%) years (Figure 5b). In "on" years, linoleic acid in 'Barnea' oils decreased to with irrigation to the 75% treatment and then increased. Linolenic acid ranged from 0.6 to 0.85% of fatty acids. In 'Souri' oils, linolenic acid increased with irrigation (Figure 5c). A similar increase was found in 'Barnea' oils in the "off" year. Stearic acid was greater in 'Souri' (3.2—3.7%) than in 'Barnea' (2.2—3.1%) oils and consistently greater in "on" as compared to "off" years (Table 1 and Figure 5d). Stearic acid increased with irrigation level in 'Souri' oil and decreased with irrigation in 'Barnea' oil. The percentage of palmitic acid (11.5—15.5%) was greater in 'Souri' than in 'Barnea' oils and was higher in "off" as compared to "on" years (Table 1 and Figure 5e). There was a slight increase of palmitic acid with irrigation in 'Barnea' in all years and in 'Souri' in the "off" year 2007. Palmitoleic acid was higher in 'Barnea' than in 'Souri' oils and was greater in "off" than in "on" years (Table 1 and Figure 5f). There was a general trend of increased palmitoleic acid with increased irrigation, which was stronger in "on" years and stronger in 'Barnea' oils (Table 1).

The ratio of saturated to unsaturated fatty acids (SAT/UNFAT) in the oil ranged from 0.18 to 0.24 (Figure 6a). SAT/UNSAT was greater for 'Souri' than 'Barnea' oils and greater in "off" than in "on" years (Table 1). The irrigation level did not consistently affect the SAT/UNSAT ratio. The contents of the PUFA were conversely correlated with the oleic acid content. The ratio of MUFA to PUFA was higher in "on" than in "off" years (Table 1 and Figure 6b). MUFA/PUFA decreased with irrigation in 'Barnea' oil in the "off" year and in 'Souri' in its "on" year. MUFA/PUFA peaked at the 75% irrigation treatment in 'Barnea' oils in the "on" years.

Squalene. The squalene content was double in 'Souri' as compared to 'Barnea' oils and was consistently lower in oils from trees receiving the lowest irrigation rates (Table 1 and Figure 7). No effect of fruit load (bearing cycle) was found on squalene content (Table 1). For 'Souri' oils, the average squalene increased as a function of increasing irrigation rate from 831 mg 100 g oil^{-1} (30% ETp) to 900 mg 100 g^{-1} (100% and 125% ETp). Squalene in 'Barnea' oils averaged 256 mg 100 g^{-1} for the 30% ETp treatment and between 380 and 390 mg 100 g^{-1} for all of the other treatments (Figure 7).

Organoleptic Tests. In oil tasting of samples extracted from the 2007 harvest of 'Barnea' and 'Souri' and 2008 of 'Souri' alone, bitterness and pungency were generally found to decrease with increased irrigation level (Figure 8). The largest effects on bitterness were found between the lowest irrigation level (30%) and that following (50%). Further increases in irrigation level lead to small reductions or no change in bitterness. The bitterness rating ranged from 3 (30% irrigation) to 0.4 and to 0.2 (50 and 125%) in 'Souri' oil in 2007, which was an '"off" year

Table 1. Means and Statistical Analysis of FFAs, Peroxide Number, Total Polyphenol Content, Fatty Acid Composition, and Squalene in Oils[a]

		2006	2007	2008	2009
		”off”	”on”	”off”	”on”
			'Barnea'		
polyphenols	mg g^{-1}	271.1 a, A	297.3 a, A	134.7 c, B	189.2 b
FFA	% oleic	1.32 a, A	0.27 b, B	0.52 b, B	0.51 b
peroxide	meq O^2 kg^{-1}	7.97 a, A	2.74 d, B	3.49 c, B	4.52 b
C 16:0	%		12.36 c, B	14.47 a, A	13.43 b
C 16:1	%		0.89 c, B	1.09 a, A	1.00 b
C 18:0	%		2.80 a, B	2.43 b, B	2.56 b
C 18:1	%		66.62 a, A	62.25 c, B	64.88 b
C 18:2	%		14.85 b, B	17.69 a, A	15.89 b
C 18:3	%		0.79 a,A	0.75 a, A	0.87 a
MUFA/PUFA			4.37 a, A	3.52 c, B	4.02 b
SAT/UNSAT			0.182 c, B	0.206 a, B	0.201 b
squalene	mg 100 g^{-1}		322.3 a, B	396.8 a, B	
			'Souri'		
polyphenols	mg g^{-1}	226.5 a, B	191.0 b, B	176.0 b, A	
FFA	% oleic	0.56 b, B	1.13 a, A	0.55 b, A	
peroxides	meq O^2 kg^{-1}	5.40 ab, B	5.63 a, A	4.94 b, A	
C 16:0 (%)	%		15.29 a, A	14.00 b, B	
C 16:1 (%)	%		0.95 a, A	0.79 b, B	
C 18:0	%		3.26 b, A	3.64 a, A	
C 18:1	%		59.25 b, B	64.08 a, A	
C 18:2	%		18.06 a, A	14.21 b, B	
C 18:3	%		0.77 a, A	0.72 b, B	
MUFA/PUFA			3.23 b, B	4.38 a, A	
SAT/UNSAT			0.234 a, A	0.222 b, A	
squalene	mg 100 g^{-1}		867.1 a, A	868.1 a, A	

[a] Unique lowercase letters indicate significant differences for parameters between the years. Unique uppercase letters indicate significance differences between varieties in same year.

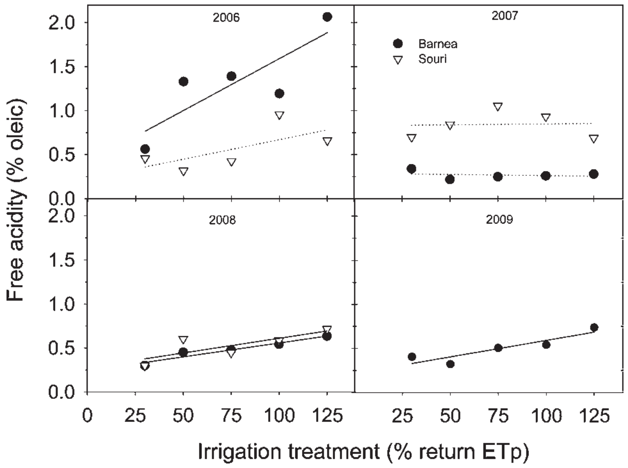

Figure 3. Free fatty acids in olive oil as a function of irrigation water application rate. Heavy fruit loads (“on” years) were experienced in 2006 and 2008 in 'Souri' trees and in 2007 and 2009 in 'Barnea'. Symbols are average measured values ($n = 10$), and lines are best-fit linear regression. Dotted lines are not significant. Regression equations and statistics are found in the Supporting Information, Tables A, D, and E.

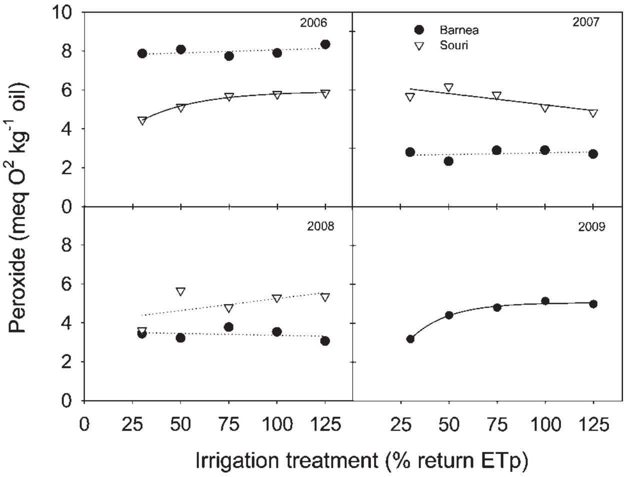

Figure 4. Peroxide content in olive oil as a function of irrigation water application rate. Heavy fruit loads (“on” years) were experienced in 2006 and 2008 in 'Souri' trees and in 2007 and 2009 in 'Barnea'. Symbols are average measured values ($n = 10$), and lines are best-fit three-parameter exponential rise to maximum or linear regression curves. Dotted lines are not significant. Regression equations and statistics are found in the Supporting Information, Tables A, D, and E.

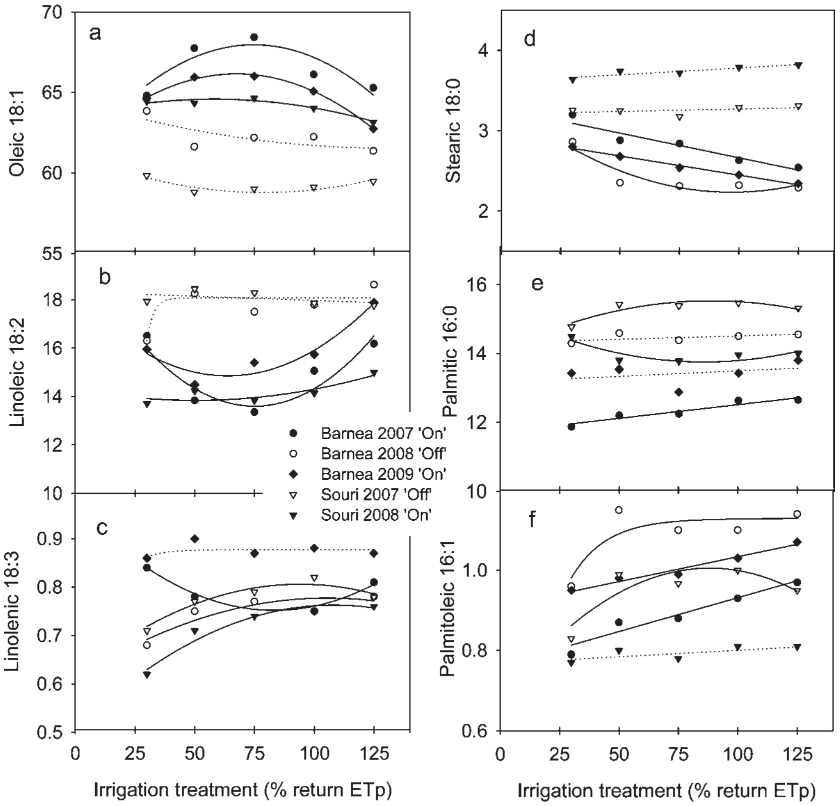

Figure 5. Fatty acid relative composition (%) in 'Barnea' cv. and 'Souri' oils for the years 2007−2009. (a) Oleic C18:1, (b) linoleic C18:2, (c) linolenic C18:3, (d) stearic C18:0, (e) palmitic C16:0, and (f) palmitoleic C16:1. Symbols are average measured values ($n = 5$, 2007; $n = 10$, 2008 and 2009), and lines are best-fit linear, quadratic polynomial, or two-parameter exponential rise to maximum regression curves. Dotted lines are not significant. Regression equations and statistics are found in the Supporting Information, Tables B, F, and G.

for the variety. Bitterness was higher in the following "on" year when it fell from 4.8 (30%) to 1.1 (50%) to 0.5 (125%). The bitterness of 'Barnea' oil in 2007, an "on" year for the variety, was higher than for 'Souri' and ranged from 6.7 at 30% irrigation to 2.3 at 100%. Pungency was also consistently highest in oil from olives irrigated at 30% of ETp. For oil from 'Souri' fruits in 2007, pungency decreased from a level of 4.5 to 0.9 between 30 and 50% treatments but then increased as irrigation increased to 1.9 at the 125% treatment. In 2008, pungency of the 'Souri' oil decreased linearly as irrigation level increased, from 4.4 (30% irrigation) to 1.8 (125% irrigation). Fruitiness was relatively high (between 4 and 6) for both varieties and in both years, and no consistent effect was found linking it to irrigation level. No negative attributes were detected in the tested oil samples.

■ DISCUSSION

The situation of the experimental orchard, with two varieties in opposite alternate bearing cycles, has provided a unique opportunity to consider irrigation application regimes in terms of fruit load. The results suggest that olives are more susceptible to reduction in oil quality parameters with increased irrigation when fruit load on a tree is low. Differences in measured values of polyphenols and FFAs found each year between varieties were obviously fruit load generated as they were found to reverse each season.

Increasing irrigation lead to fruits with greater water content as well as to oil with relative decreases in polyphenol content (Figure 2) and frequent increased FFAs (Figure 3). The connection between water stress and higher polyphenols is well established. Artajo et al.[34] proposed that the water status of trees affects phenol production in olive fruit and consequently the phenol content of the olive paste. They and others[10,16] suggested that water or other[35] stressors trigger augmented phenol synthesis. It has alternatively been hypothesized that phenolic compounds, rather than being produced at lower rates, are partitioned during the olive oil extraction process and removed at greater rates with the water separated from oil in the mill.[36,37]

The findings regarding higher FFA in "off" years and increased FFA content with increased irrigation levels are less apparent in the literature. The vast majority of studies find no or little effect of irrigation level on total acidity or on fatty acid profiles, while a few[23,15,38] do indicate increases in FFA with increased irrigation. The inconsistency regarding these findings, and the fact that the cases of increased FFA with increased irrigation have been found mainly outside the traditional center of olive production in southwestern Europe, may indicate that the phenomenon is dependent on climate and variety. The positive correlations between low fruit load and FFA content and between irrigation and FFA content could be due to increased sensitivity of fruit with higher water content and thinner cuticle layer to mechanical injury.[11,15] The relatively high levels of oleic acid in extra virgin olive oil have been shown contribute to reduction of oxidation.[39,40] Therefore, the higher oleic acid (Figure 5 a) found in oil from "on" trees is expected to additionally contribute to the oxidative stability of oils and their lower FFA content.

On several occasions, FFA values were particularly high even after consideration of irrigation and bearing levels (Figure 3, 'Barnea' 2006 and 'Souri' 2007). Episodes of such high FFA occur

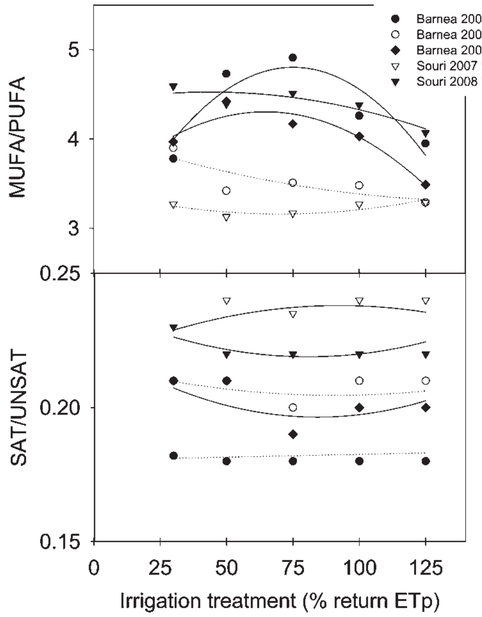

Figure 6. Fatty acid indexes for 'Barnea' cv. and 'Souri' oils for the years 2007−2009. (a) Monounsaturated to polyunsaturated ratio (MUFA/PUFA) and (b) saturated to unsaturated fatty acid ratio (SAT/UNSAT). Symbols are average measured values ($n = 5$, 2007; $n = 10$, 2008 and 2009), and lines are best-fit linear or quadratic polynomial regression curves. Dotted lines are not significant. Regression equations and statistics are found in the Supporting Information, Tables B, F, and G.

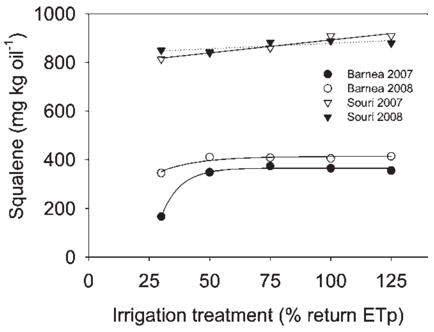

Figure 7. Squalene content in 'Barnea' cv. and 'Souri' oils for the years 2007 and 2008. Symbols are average measured values ($n = 5$, 2007; $n = 10$, 2008), and lines are best-fit linear, two- or three-parameter exponential rise to maximum regression curves. Dotted lines are not significant. Regression equations and statistics are found in the Supporting Information, Tables B, F, and G.

from time to time in olive oils from this region. The reason for this is unknown, although climate, pests, and diseases have been suggested. Regarding effects from pests, a good deal of attention has been focused on olive fruit fly (*Bactrocera oleae*) as a possible cause for olive oil deterioration.[41] However, because this pest was consistently controlled in the orchard and infestation level throughout the experimental years did not exceed 2%, it was likely not a major cause for the high FFA levels in this case. Another possible cause for general high FFA is infestation with anthracnose (*Colleototrichum acutatum* and *Colleototrichum gloeosporioides*).[42] The seasons with high FFA were characterized by relatively high humidity, which could accelerate the infestation with these fungi.

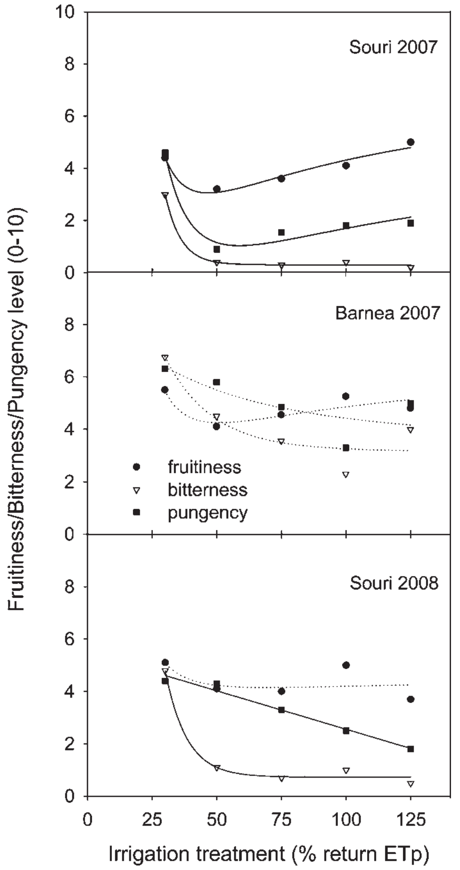

Figure 8. Panel results for sensory organeleptic evaluation for oils from 2007 harvest ('Barnea' and 'Souri') and from 2008 ('Souri'). In 2007, 'Souri' trees were “off” and 'Barnea' trees “on” and in 2008 the 'Souri' trees were “on”. Dotted lines are not significant. Regression equations and statistics are found in the Supporting Information, Table C.

While we have no quantitative data supporting correlation between fungal infestation and FFA, we conjecture that fruit with higher water content and lower polyphenol content would be at greater risk of fungal inoculation.

We found greater oleic and palmitoleic acids in 'Barnea' oils and greater stearic and palmitic acids in 'Souri' oils (Table 1). The “on” years consistently had higher percentages of oleic and stearic acids and lower percentages of linoleic, palmitic, and palmitoleic acids as compared to the “off” years (Table 1). Beltrán et al.[43] previously demonstrated that yield level was the dominant factor in determination of fatty acid composition in 'Picual' oils and that oleic acid was higher when yield was greater. Increased irrigation was accompanied by increased percentages of palmitoleic, palmitic (all years 'Barnea', “off” year 'Souri'), and linoleic acid (all years 'Souri', “off” years 'Barnea'). Stearic acid increased with irrigation in 'Souri' oils and decreased in 'Barnea' oils. The fact that a midlevel (slightly deficit) irrigation regime produced significantly higher oleic acid in “on” years for both varieties is unique. The health-promoting effects achieved by consumption of high oleic olive oils oil could be due to enrichment of low-density lipoprotein (LDL) with oleic acid. Also of interest are the trends of increased palmitoleic 16:1, palmitic 16:0, and linolenic 18:3 acids with irrigation since most of the data found in the literature do not indicate any effect of irrigation on olive oil fatty acid composition.[25,16,11,13] Gomez-Rico et al.[13] did find that oleic acid content and MUFA/PUFA ratio were significantly higher in oils obtained from rain-fed olives as compared to oils from irrigated olives. The changes found in the levels and ratios of the fatty acids, corresponding to applied water and fruit load, may limit the potential of these values to serve for the geographic determination of the origin of olive oils and should be followed carefully in irrigated orchards to ensure compatibility with trade standards. Moreover, the dramatic changes in climate in the Mediterranean basin, both current and predicted, may further confuse the concept of specific (and constant) oil characteristics for each geographical region.

Squalene, one of the more potent, oil soluble, edible antioxidants, is well absorbed by mammals, protects against various oxidative stress related diseases, and acts as an anticarcinogenic and antiflamatory agent. Sources for squalene in the human diet are limited, and olive oil has been indicated as an available, rich dietary supply.[44] The squalene level in olive oil has been shown to decrease as a function of ripening level.[45] We found that the squalene level was cultivar but not seasonally dependent. The observation that squalene was reduced under water stress and was consistently higher in oils of well irrigated olives is novel and of interest due to squalene's contribution both to human health upon consumption[46] and to oil shelf life.[47]

Organoleptic evaluation showed that sensory levels of bitterness and pungency closely followed total polyphenol content measurements in the oil. The polyphenol relationship with bitterness and pungency of olive oil is well established[48,49] and has been previously presented in irrigation experiments.[50,51,23]

The parameters measured in this study and interesting to growers are expected to be related to cultivar and environment and are highly influenced by bearing level and cycles. The unique case in this study where two varieties were in opposite alternate bearing cycles has allowed interpretation of the relative importance of water availability in terms of biannual bearing cycles and fruit load. Absolute values of variables in this study, including polyphenol content, FFA, and oleic acid, were highly correlated to fruit load and secondarily were a function of irrigation level. The additive contribution of polyphenols, squalene, and oleic acid to both the shelf life of the oil and the health benefits associated with its consumption suggests that these compounds should be considered when making decisions regarding cultivar selection and orchard management.

Other studies have shown conflicts between olive oil yield and quality relating to water application,[52,17,53,54] indicating that deficit irrigation regimes are likely to optimize oil production. Water stress management for target oil quality is becoming more popular and seems to become a likely aspect of orchard management, especially in high density orchards and orchards where relatively high irrigation application rates are dictated to avoid excessive build-up of salts in the root zone (as with saline and wastewater irrigation). It is likely that regulated, temporal, deficit irrigation during certain olive growth stages will eventually target desired water stress levels in attempts to secure quality levels without harming yields. Efforts are currently being made to develop tools to monitor and maintain the target stress levels.[7,55−57]

The results suggest a number of likely methods to benefit irrigation management including irrigation regimes that are cultivar specific and irrigation systems that cater to each variety individually. Stress levels and water requirements are highly dependent on fruit load, and the best irrigation management must account for biannual bearing effects. More work is necessary to confirm these data over an even longer term, to determine optimum water stress scheduling (stress levels and timing) for best yield−quality combinations, and to further develop methods for monitoring and maintaining water stress levels.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Regression equations and statistics for Figures 1−8. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 972(0)8 9928644. Fax: 972(0)8 9926485. E-mail: bengal@volcani.agri.gov.il.

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